Amendment to the Specification

At the indicated page and line numbers, please replace the existing paragraphs with the paragraphs that follow.

(Page 22, line 11 through page 23, line 4)

Malignant transformation is believed to be a multi-step process and chromosomal translocations that generate chimeric proteins such as EWS/ATF1 may initiate a cascade of events leading to cancer ~~(Arevalo et al., 1993; Gao and Paul, 1995; and Glockshuber et al., 1992)~~ (Bridge et al., 1990; Kroemer et al., 1998). The exquisite specificity of antibodies for defined targets presents numerous opportunities for disrupting protein-protein or protein-DNA interactions, particularly when the targeted structures are complex and not amenable to blockade by small molecules. Recently, scFvs have been used to achieve phenotypic knockout of cell surface or cytoplasmic target proteins involved in neoplasia such as Ki-ras, ErbB2, epidermal growth factor receptor and the IL2 receptor (Marasco, 1995; Duan et al., 1995; Graus-Porta et al., 1995; Griffiths et al., 1993). As an embodiment of the present invention, it was discovered that a similar approach could be used to disrupt activity of a nuclear protein and demonstrate its role in the neoplastic process. In SU-CCS-1 cells, interference with the activity of EWS/ATF1 could theoretically eliminate the initiating process leading to neoplasia and yet have no effect on tumor growth since other pathways may become dominant following transformation. Interference with DNA binding and transcriptional activity by the ATF1-inhibitory sFv demonstrated EWS/ATF1 is important for maintenance of tumor cell viability in addition to its previously proposed role in initiating the neoplastic process ~~(Hileman et al., 1994)~~ (Ohno et al., 1994). Although DNA binding was blocked,

the EWS/ATF1 protein remained available for interactions with other proteins of the transcriptional apparatus ~~(Churchill et al., 1994)~~ (Paula et al., 1999).

(Page 23, lines 5 through 15)

The predicted interactions between CRE DNA and ATF1 are based on the structural studies of GCN4 bound to CRE DNA by Richmond and Keonig ~~(Grim, et al., 1996; and Hage and Twee, 1997)~~ (Konig et al., 1993; Ellenberger et al., 1992). A conformational change in a linker domain of EWS/ATF1 may occur following binding by sFv4, or presence of the antibody may destabilize the important amino a cid side chain interactions with the phosphate-DNA backbone. When EWS/ATF1 is not bound to DNA, the antibody may prevent binding of transcription factor to DNA by occupying a region adjacent to the DNA binding domain. Although the binding kinetics of EWS/ATF1 are not known, sFv4 has been shown to disrupt ATF1-DNA complexes, and the presence of sFv4 in the region between the a helices may also prevent rebinding of the factor to DNA. If immunodepletion is the mechanism, then the inhibitory effect of sFv4 on EWS/ATF1 may be due to the removal of transcription factor from the cellular pool by altering its intracellular processing or nuclear transport.

(Page 23, line 16 through page 24, line 16)

Fujimura (1996) has proposed that EWS is a negative regulator of ATF1 binding activity based on relatively lower intensity of recombinant protein complexes in gel shift assays and results from deletion mutant experiments ~~(Fisher and Fivash, 1994)~~ (Fujimura, et al., 1996). We also noted a significant difference in the relative binding affinity of recombinant EWS/ATF1 to the CRE as compared with recombinant ATF1 when measured by band intensity on EMSA. However, the intensity of EWS/ATF1-CRE complexes using cellular extracts

from either 293T or SU-CCS-1 cells was roughly equivalent to that seen with recombinant ATF1. Therefore post-translational modification of EWS/ATF1 may be important for regulating binding activity as has been shown for EWS/FLI ~~(Hai et al., 1988)~~ Deloulme et al., 1997). In direct comparison with ATF1, EWS/ATF1 greatly increases gene expression when measured by reporter assay ~~(Fisher and Fivash, 1994; and Hileman et al., 1994)~~ (Fujimura et al., 1996; Ohno et al., 1994). The increased expression with EWS/ATF1 is thought to result from either the loss of regulatory elements by truncation of ATF1 or the contribution of the potent EWS transcription activation domain ~~(Chothia and Lesk, 1987)~~ (Li and Lee, 1998). A quantitative comparison of EWS/ATF1 to other intracellular proteins in human tumors has not been previously demonstrated. Since the chimeric protein is not produced in the absence of the translocation between chromosomes 12 and 22, expression levels must be compared with other endogenous protein. As determined by cytogenetic analysis, a single allele of the wild type EWS and ATF1 genes remains intact in SU-CCS-1 cells. Our western blot experiments indicate that EWS/ATF1 is present in considerable excess to the endogenous levels of ATF1 in the SU-CCS-1 cell line and a CCS tumor. Densitometric analysis indicated that EWS/ATF1 is expressed at a 3.0 fold greater level than ATFI in the SU-CCS-1 cell line and a 10.6 fold greater level in a CCS tumor. As originally suggested for Ewing's sarcoma, the EWS/ATF1 fusion protein may achieve transformation through both over-expression and strong transcriptional activation capability ~~(Jameson and Sawyer, 1980)~~ (Peterman et al., 1998). Similar explanations have been proposed for alveolar rhabdomyosarcoma associated with translocations of the PAX3 and FKHR protein genes ~~(Kabat et al., 1992)~~ (Barr, 1997).

(Page 24, line 17 through page 25, line 5)

EWS/FLI, EWS/ATF1 and other chimeric proteins resulting from specific translocations in leukemias, lymphomas and sarcomas can be considered true tumor-specific proteins and the linker domain can serve as a unique epitope for derivation of antibodies. However, molecular modeling of the EWS/ATF1 chimeric protein suggested that the fusion junction was not an exposed surface and unlikely to be available for binding by antibody. As demonstrated with mAb5 (Example 9), binding of transcription factors by antibody does not necessarily result in loss of function *in vitro*. Intracellular expression of sFv4 reduced activity of the CRE containing proliferating cell nuclear antigen (PCNA) promoter by approximately 60%, but no loss of cell viability was seen when compared to controls ~~(Darsley et al., 1985)~~ <u>(Bosilevac etal., 1998)</u>. HeLa cell transfections were performed and verified that sFv4 expression was not cytotoxic in cells without EWS/ATF1. No loss in viability was observed in transfected HeLa cells which suggests that sFv4 induced cell death in SU-CCS-1 cells by disruption of EWS/ATF1 activity and not through inhibition of endogenous ATF1 activity.

(Page 25, lines 6 through 11)

The process of cell death in SU-CCS-1 cells exposed to sFv4 appears to have occurred through an apoptopic mechanism ~~(Fisher et al., 1993)~~ <u>(Gavrieli et al., 1992)</u>. The finding that 30% of cells exposed to SRα-Fv4 were apoptotic as compared to controls ($p<0.005$) is comparable to results observed by others in studies of apoptosis ~~(Koike et al., 1989; and Konig and Richmond, 1993)~~ <u>(Boehm et al., 1998)</u>. However, cell death involves multiple pathways and ultra-structural studies are helpful in determining whether evidence of necrosis is present ~~(Gao and Paul, 1995)~~ <u>(Kroemer et al., 1998)</u>.

(Page 25, lines 12 through 22)

Disruption of key molecular processes responsible for neoplastic transformation and reversal of malignant phenotypes are important goals in developing new cancer therapeutics ~~(Kubota et al., 1996)~~ (Anderson, 1998). The targeted disruption of EWS/ATF1 activity via the ATF1 epitope of sFv4 reduced SU-CCS-1 cell viability but had little effect on HeLa cells not expressing the oncogenic fusion protein. By demonstrating activity in this tumor cell type, we demonstrate the importance of chimeric proteins with transcriptional activity in maintenance of tumor cell viability. The evidence presented here has broad application to leukemias, lymphomas and other sarcomas with characteristic chromosomal translocations involving transcription factors such as the EWS/FLI-1 in Ewings Sarcoma and PAX3/FKHR in alveolar rhabdomyosarcoma. Because the level of the oncogenic EWS/ATF1 protein is higher in primary tumors than in established cell lines, and *in vivo* studies would be appropriate to determine the therapeutic potential for disruption of fusion protein transcriptional activity by antibodies.

(Page 26, lines 4 through 14)

Ewing's sarcoma and PNET are tumors of childhood and adolescence with a consistent chromosomal translocation ~~(Busch et al., 1990; and Ellenberger et al., 1992)~~. Ewing's Sarcoma and PNET are related if not the same tumor type and one observation supporting a common origin is the characteristic translocation involving the Ewing's sarcoma protein (EWS) and the Friend leukemia integration site I protein (FLI1) (May et al., 1993). The translocation results in the generation of a chimeric gene that joins the 5' portion of the EWS locus to the 3' region of the FLI1 gene resulting in the replacement of the transcription activation domain of FLI1 with EWS. This

chromosomal translocation is found in over 90% of Ewing's sarcoma and PNETs, strongly suggesting the product of this rearrangement is critical for the development of these malignancies (Ladanyi, 1995). The reciprocal translocation does not result in an expressed protein due to the presence of an in-frame stop codon immediately C-terminal to the FLI1 sequence.

(Page 29, line 14 through page 30, line 3)

The human paired box (PAX) genes compose a family of transcription factors that play a fundamental role in the regulation of development such as the kidneys and genital tracts (PAX 2) B cells (PAX 5), eye structures (PAX 6) and muscle development (PAX3 and PAX 7) ~~(Hinrichs et al., 1984)~~ (Strachan et al., 1994). Following muscle cell differentiation both PAX 7 and 3 are down-regulated. PAX 3 is also implicated in the migration of muscle cell precursors suggesting a critical role in myogenesis. The forkhead family of transcription factors includes FKHR however the specific contribution of FKHR to oncogenesis is uncertain. Recently, deletion studies of PAX/FKHR have shown that mutations of the FKHR activation domain are unable to transform NIH 3T3 cells (Lam et al., 1999). Therefore, FKHR is thought to contribute to oncogenesis through its effect on protein-protein interactions of factors involved in transcription. PAX proteins and other proteins involved in cell differentiation and normal development are expressed at specific time points in cell development and are subsequently down regulated in conjunction with differentiation. Therefore, interference with their endogenous activity in fully differentiated cells may not have untoward biological effect.